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[RAPID COMMUNICATION]

Absence of Methylated Thyrotropin-Releasing Hormone in the Bullfrog (*Rana catesbeiana*) Brain

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ABSTRACT—The recent finding that [1-Me-His^2]TRH is present in the carp brain prompted us to ascertain whether methylated TRH also exists in the frog brain. Samples were prepared from whole brain tissues of prometamorphic and climax tadpoles, and juvenile and adult frogs by acid extraction. Each sample was subjected to reverse-phase high-performance liquid chromatogrphy. The eluates were assayed for TRH using two kinds of antibodies against TRH, one showing 100% cross-reactivity with both methylated TRHs and the other showing 3% and 43% cross-reactivities with [1-Me-His^2]TRH and [3-Me-His^2]TRH, respectively. Both the distribution and amount of immunoreactive TRH in the eluates of each sample did not vary between the assays using the two different antibodies. In both cases, a single peak with a similar immunoreactivity appeared at a position similar to that of synthetic TRH. Thus, it is concluded that brains of larval and adult bullfrog contain no appreciable amount of methylated TRH.

INTRODUCTION

In amphibians, thyrotropin-releasing hormone (TRH, pGlu-His-Pro-NH_2) is known to have potent prolactin (PRL)-releasing activity [1, 2, 6, 9], as in mammals [12] and is regarded as the major PRL-releasing factor present in the bullfrog hypothalamus [7, 10]. Recently, the presence of [1-Me-His^2]TRH has been reported in the brain of cyprinoid fish [3, 5]. In amphibians, [1-Me-His^2]TRH has no PRL-releasing activity [8]. Another [3-Me-His^2]TRH is known to have biological activity ten-fold higher than that of TRH in mammals [13]. Potent PRL-releasing activity of [3-Me-His^2]TRH has also been confirmed in the bullfrog (*Rana catesbeiana*) [8]. These findings prompted us to determine whether methylated tripeptides exist in bullfrog brain.

MATERIALS AND METHODS

Peptides

TRH and [3-Me-His^2]TRH were purchased from Sigma and Bachem Inc., respectively. [1-Me-His^2]TRH was synthesized in the laboratory of one of the authors (T.Y.) [5].

TRH-antisera

Two kinds of TRH-antisera (AS1 and AS2) produced in rabbits by immunizing with TRH complexed to bovine serum albumin [4, 11] were used. AS1 showed 100% cross-reactivity with both methylated TRHs. AS2 showed 3% and 43% cross-reactivity with [1-Me-His^2]TRH and [3-Me-His^2]TRH, respectively.

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Acid extract of brain tissue

Whole brain tissue without the hypophysis from 220 prometamorphic tadpoles, 137 climax tadpoles, 36 juvenile frogs and 23 adult frogs captured in September were dissected out, immudiately frozen on dry ice and stored at −80°C until use. They were homogenized separately in cold 0.1 N HCl at 3,000 rmp for 30 min and centrifuged at 22,000×*g* for 60 min. The precipitate was re-homogenized in the same solution and centrifuged. The resulting supernatants were combined, neutralized with NaOH, centrifuged at 22,000×*g* for 60 min and lyophilized.

Reverse-phase high-performance liquid chromatography (RP-HPLC)

Each lyophilized brain extract was dissolved in

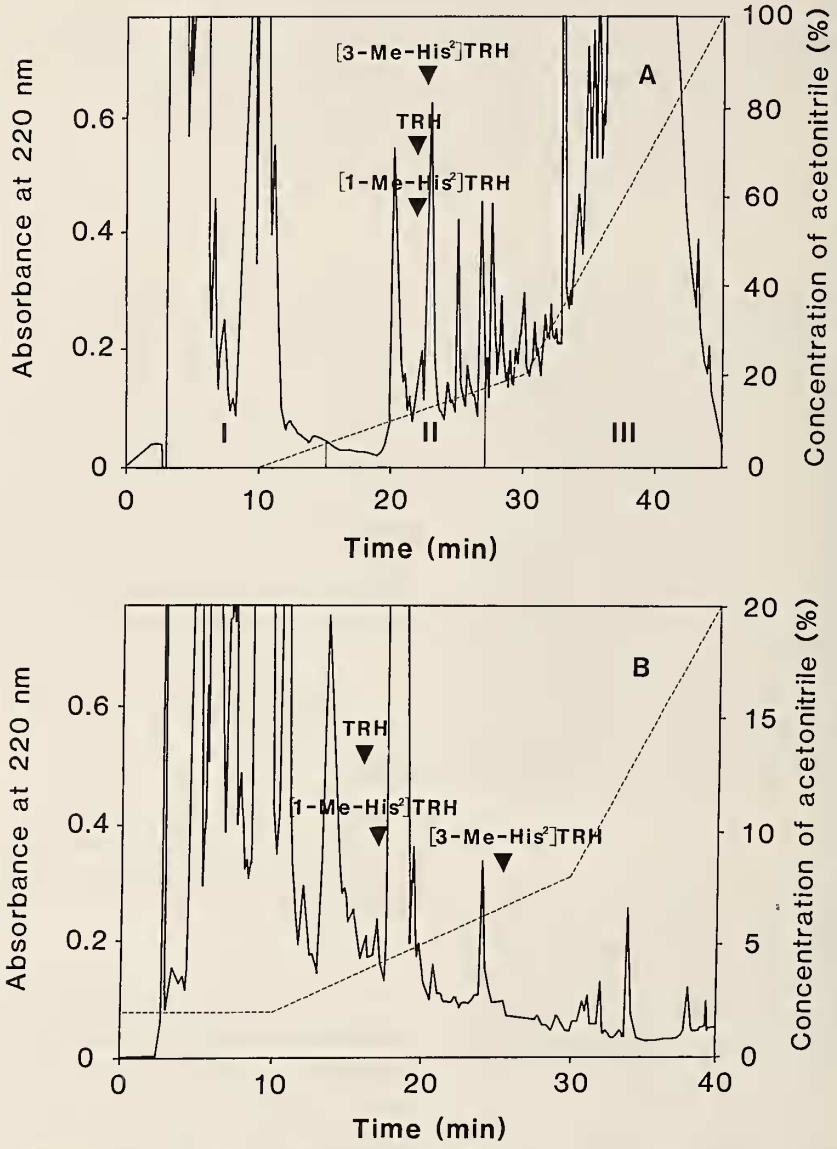

FIG. 1. Reverse-phase HPLC of acid extract of adult frog brain (A) and of fraction II obtained from adult brain extract (B).

Milli-Q water, and subjected to HPLC (LC-800; JASCO) on a reverse-phase column (TSK-gel ODS-120T; TOSOH) at a flow rate of 1 ml/min. Gradient elution was carried out for 35 min with 0–100% acetonitrile containing 0.1% trifluoroacetic acid (Fig. 1A). The elute was monitored by measuring the absorbance at 220 nm. The fractions were pooled as three separate fractions and lyophilized. These fractions were assayed for TRH using AS1. A fraction containing immunoreacitve TRH was further subjected to HPLC an a TSK-gel ODS-120T column equilibrated with 20 mM phosphate buffer (pH 6.5) (solution A) at a flow rate of 1 ml/min. Gradient elution was carried out for 30 min with solution A containing 10–100% solution B (20% acetonitrile in Milli-Q water) (Fig. 1B). A mixture of standard TRH, [1-Me-His^2]TRH and [3-Me-His^2]TRH or TRH and [1-Me-His^2]TRH was also subjected to HPLC. The eluate was monitored by measuring the absorbance at 220 nm, and each 1-ml fraction was collected.

Radioimmunoassay

Each fraction was assayed for TRH using AS1 and AS2, according to the method of Winokur and Utiger [14].

RESULTS AND DISCUSSION

At present, no specific antisera for detecting [1-Me-His^2]TRH and [3-Me-His^2]TRH are available. In the present experiment we employed radioimmunoassay using two kinds of anti-TRH sera showing different cross-reactivity with [1-Me-His^2]TRH and [3-Me-His^2]TRH in addition to reverse-phase HPLC. When the mixture of synthetic TRH, [1-Me-His^2]TRH and [3-Me-His^2]TRH was subjected to HPLC, the amount of immunoassayable TRH in each 1-ml fraction differed according to the antiserum used (Fig. 2A). When the ratio of [1-Me-His^2]TRH to TRH was reduced from 1:4 to 1:8, the amounts of radioimmunoassayable TRH detectable with AS1 and with AS2 scarcely differed (data not shown).

The fraction containing immunoassayable TRH (Fraction II) was separated from larval and adult brain tissue by the first step of HPLC (Fig. 1A). The amounts of brain tissue which yielded 100 ng of immunoassayable TRH in prometamorphic and climax tadpoles and juvenile and adult frogs were 45.3, 40.0, 15.3 and 10.3 mg (wet weight), respectively. Each fraction containing 100 ng of immunoassayable TRH was subjected to the final HPLC (Fig. 1B). Immunoassayable TRH eluted at the theoretical positions for TRH and [1-Me-His^2]TRH did not differ according to the antiserum used (Fig. 2B-E), indicating that neither larval nor adult brain contains an appreciable amount of [1-Me-His^2]TRH. No immunoreactivity was found at the theoretical position for [3-Me-His^2]TRH. It is concluded that in the bullfrog

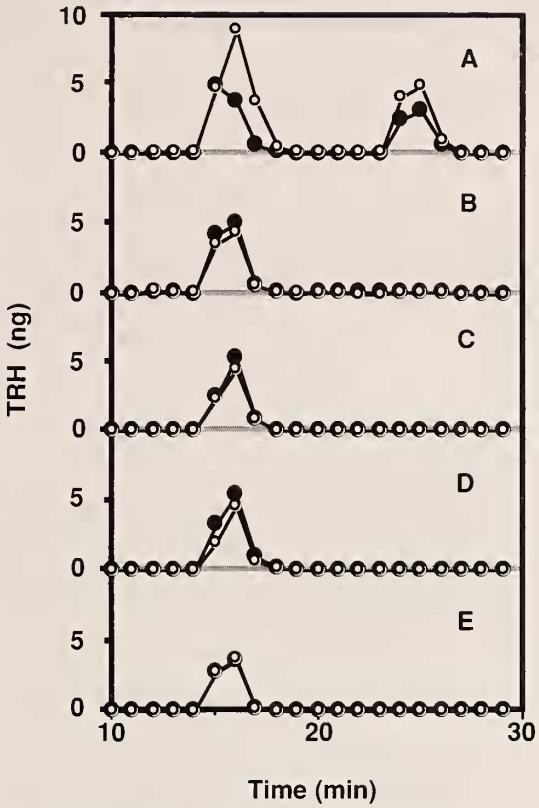

FIG. 2. The amount of immunoreactive TRH in 100 μl of each 1-ml fraction obtained by RP-HPLC. A: Mixture of 100 ng each of synthetic TRH, [1-Me-His^2]TRH and [3-Me-His^2]TRH. B: Extract of the tadpole brain at prometamorphosis (stages 12–19). C: Extract of the tadpole brain at the climax stage (stages 20–24). D: Extract of the juvenile frog brain. E: Extract of the adult frog brain. ○: RIA with AS1. ●: RIA with AS2.

brain no measurable amount of methylated TRH exists, although the possibility that methylated TRH appears at a specific developmental stage or in a specific season cannot be excluded.

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REFERENCES

1 Clemons GK, Russell SM, Nicoll CN (1979) Gen Comp Endocrinol 38: 62–67
2 Hall TR, Chadwick A (1984) J Endocrinol 102: 175–180
3 Hamano K, Inoue K, Yanagisawa T (1990) Gen Comp Endocrinol 80: 85–92
4 Hamano K, Sato A, Yanagisawa T (1989) Nippon Suisan Gakkaishi 55(10): 1741–1745
5 Hamano K, Tamura S, Yanagisawa T (1990) Nippon Suisan Gakkaishi 56(2): 365
6 Kikuyama S, Seiki T (1983) In "Neuropeptides, Neurotransmitters and Regulation of Endocrine Processes" Ed by E Endoroczi, Academial Kiado, Budapest, pp 317–324
7 Nakajima K, Uchida D, Sakai M, Takahashi N, Yanagisawa T, Yamamoto K, Kikuyama S (1993) Gen Comp Endocrinol 89: 11–16
8 Sakai M, Uchida D, Yanagisawa T, Kikuyama S (1991) Zool Sci 8: 935–937
9 Seki T, Kikuyama S (1986) Gen Comp Endocrinol 61: 197–202
10 Seki T, Kikuyama S, Suzuki M (1988) Zool Sci 5: 407–413
11 Taniguchi Y, Tanaka S, Kurosumi K (1990) Zool Sci 7: 427–433
12 Vale W, Rivier C, Brown M (1977) Ann Rev Physiol 39: 473–527
13 Vale W, Rivier J, Burgus P (1971) Endocrinology 89: 1485–1488
14 Winokur A, Utiger RD (1974) Science 185: 265–267